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(54) Title: DIAGNOSTIC METHODS

(57) Abstract: The present invention derives from the identification of 26 gene transcripts (Markers) that exhibit aberrant expression levels in prostate disorder tissues. The invention therefore relates to diagnostic techniques for the detection of human prostate disorders, such as cancer, by detecting one or more of these Markers, intermediates, precursors or products (mRNA, cDNA, genomic DNA, or protein). The invention is also directed to methods for identifying modulators of prostate disorders, which modulators, such as chemical compounds, antisense molecules and antibodies interact with and modulate any one of the Markers identified.

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In early disease, where the cancer is confined to the prostate gland and has not spread beyond the gland, the tumour can potentially be removed by surgery. Surgery is a common treatment of prostate cancer. Transurethral resection is a procedure in which the cancer is cut from the prostate via the urethra. A radical prostatectomy is where the whole prostate and some of the tissue around the gland is removed. Radiation therapy is also used to treat early prostate cancer.

Once disease has become metastatic, its course is rapid and relatively predictable, leading to death within 2-5 years if untreated.

Prostate cancer commonly occurs in men above the age of 50 years. The incidence of prostate cancer rises sharply between the ages of 60 and 80 years, with more than 80% of all cases of the diseases being diagnosed in men over 65 and less than 1% in men under 50 years of age. The incidence and prevalence of prostate cancer have increased considerably throughout the world over the past two decades. The average age of detection is 72 in the UK and 66 in the US. Active screening programmes may lead to a lowering of the average age at first diagnosis.

Routine medical examinations for obstructive urinary symptoms may lead to an initial diagnosis of prostate cancer. When prostate cancer is asymptomatic, there are two tests that can be performed to detect the presence of cancer: digital rectal examination (DRE) and prostate specific antigen (PSA). PSA is an enzyme which is secreted almost exclusively by epithelial cells in the prostate; measurement of the quantity of PSA in the blood provides a more reliable indicator of the presence of prostate cancer than the DRE test. However, elevations in serum levels of PSA can also be found in patients with benign prostatic hyperplasia (BPH) and prostatitis. In some cases, where a patient has elevated levels of PSA in his blood, a third diagnostic technique is used called 'transrectal ultrasonograpy' (TRUS), which can further substantiate the presence of prostate cancer. In conjunction with taking a biopsy of the tissue, TRUS has been shown to increase the detection rate of prostate cancer two-fold, when compared to DRE alone. The combination of digital rectal examination, serum PSA level and transrectal ultrasound is currently the best available diagnostic tool (Gorgoulis VG. et al. Anticancer Res 19:2327-2348 (1999)).

It is common for the prostate gland to become enlarged with ageing, a condition called benign prostatic hyperplasia (BPH), or benign prostatic hypertrophy. Severe BPH can cause

nucleic acid targeted to the Marker mRNA; or more widely in the identification or development of chemical or hormonal therapeutic agents. The person skilled in the art is also capable of devising screening assays to identify compounds (chemical or biological) that modulate (activate or inhibit) one or other of the identified Marker, which compounds may prove useful as therapeutic agents in treating a prostate disorder. Monitoring the Markers could also be useful in identifying inhibitors, antagonists or biochemical signalling effects in high throughput screening.

According to a first aspect of the invention there is provided a method for diagnosing or prognosing or monitoring a prostate disorder comprising testing a biological sample for aberrant levels of one or more of the Markers selected from the group consisting of Marker 1 to Marker 26. Preferred Markers are numbers: 8, 13, 14, 15, 17, 18, 19, 20, 22, 24, 25 and 26.

The invention lies in the identification of these differentially expressed Markers in prostate disorders. Accordingly, the invention is directed to any diagnostic method capable of assessing the differential expression levels, relative to expression in control tissues, of one or more of the identified Markers, either alone or as a panel. In particular, such methods include assessment of mRNA transcript levels and/or protein levels. The presence of aberrant expression levels of one of the Markers indicating the presence of a prostate disorder.

In a preferred embodiment the diagnostic method involves testing for more than one of the Markers identified herein. As separate independent embodiments the diagnostic method may involve testing for 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or all of the Markers identified herein, optionally as part of a panel test of other gene Markers.

It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the amount of any of the Marker products present in a test sample.

The test sample comprising nucleic acid or protein is conveniently any prostatic material or biological sample, including TURP chip, biopsy, excised prostate or part thereof, a sample of bone marrow aspirate, bone marrow biopsy, lymph node aspirate, lymph node biopsy, spleen tissue, fine needle aspirate, skin biopsy or organ tissue biopsy, particularly prostate gland tissue, invaded surgical margin, invaded lymph node, invaded lung, invaded

biomarkers for distinguishing cancer from BPH. Highly specific yet low abundance Markers could be invaluable if secreted in protein form, as they could permit non-invasive testing of body fluids.

According to a further aspect of the invention there is provided a method for distinguishing prostate cancer from BPH comprising testing a biological sample for aberrant levels of one or more of the Markers selected from the group consisting of Marker 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 17, 18, 20, 23, 25 and 26. Such a method might involve measuring the expression levels of one or more of these Markers in a biological sample taken from an individual, comparing the Marker expression level detected with control values or historical values and depending on the value detected determining whether or not the individual has prostate cancer or BPH.

Each of the Marker genes useful in the present invention are already in the public domain. However, the inventors are not aware of any prior art disclosing an association of any of the Marker genes with prostate cancer. Thus, although each of the Markers/genes identified herein are themselves already known, their association with prostate disorders leading to the present invention is unknown.

Marker 1 is the human smooth muscle protein, 22kDa. The gene coding for this protein has been cloned and sequenced (Thweatt et al., Biochem. Biophys. Res. Commun. 187:1-7 (1992)). These authors identify 22kD smooth muscle protein as a fibrobast or smooth muscle protein. There is no teaching in this paper of an association with prostate cancer. The sequence of 22kDa smooth muscle protein is disclosed in Thweatt et al. and is present in the EMBL database under accession number HS22SM. For the purpose of this application, the gene and amino acid sequences and sequence positions referred to herein, refers to that in the EMBL database under accession number HS22SM, unless stated otherwise or apparent from the context.

Marker 2 is the Rho GDP dissociation inhibitor protein. The gene coding for this protein has been cloned and sequenced (Leffers et al., Exp. Cell Res. 209:165-174 (1993)). These authors identify Rho GDP dissociation inhibitor protein as a keratinocyte protein. There is no teaching in this paper of an association with prostate cancer. The sequence of Rho GDP dissociation inhibitor protein is disclosed in Leffers et al., and is present in the EMBL database under accession number HSRHO1. For the purpose of this application, the gene and amino acid sequences and sequence positions referred to herein, refers to that in the EMBL

acid sequences and sequence positions referred to herein, refers to that in the EMBL database under accession number HSD966, unless stated otherwise or apparent from the context.

Marker 7 is the human mRNA KIAA0120. This cDNA has been cloned and sequenced (Nagase et al., DNA Res. 2:37-43 (1995)). These authors identify KIAA0120 as a brain transcript. There is no teaching in this paper of an association with prostate cancer. The sequence KIAA0120 is disclosed in Nagase et al., and is present in the EMBL database under accession number HSORFF. For the purpose of this application, the gene and amino acid sequences and sequence positions referred to herein, refers to that in the EMBL database under accession number HSORFF, unless stated otherwise or apparent from the context.

Marker 8 is the human ribosomal protein S25. The gene coding for this protein has been cloned and sequenced (Li et al., Gene 107:329-333 (1991)). These authors identify ribosomal protein S25 as a leukaemia cell line protein. There is no teaching in this paper of an association with prostate cancer. The sequence of human ribosomal protein S25 is disclosed in Li et al., and is present in the EMBL database under accession number HSRPS25. For the purpose of this application, the gene and amino acid sequences and sequence positions referred to herein, refers to that in the EMBL database under accession number HSRPS25, unless stated otherwise or apparent from the context.

Marker 9 is the human 80K-H protein (kinase C substrate). The gene coding for this protein has been cloned and sequenced (Sakai et al., Genomics 5:309-315 (1989)). These authors identify 80K-H protein (kinase C substrate) as a fibroblast and epidermal carcinoma cell protein. There is no teaching in this paper of an association with prostate cancer. The sequence of 80K-H protein (kinase C substrate) is disclosed in Sakai et al., and is present in the EMBL database under accession number HSG19P1A. For the purpose of this application, the gene and amino acid sequences and sequence positions referred to herein, refers to that in the EMBL database under accession number HSG19P1A, unless stated otherwise or apparent from the context.

Marker 10 is the human alpha-2-macroglobulin protease inhibitor protein. The gene coding for this protein has been cloned and sequenced (Kan et al., Proc. Natl. Acad. Sci. U.S.A. 82:2282-2286 (1985)). These authors identify alpha-2-macroglobulin protease inhibitor protein as a serum protein. There is no teaching in this paper of an association with prostate cancer. The sequence of alpha-2-macroglobulin protease inhibitor protein is disclosed in Kan et al., and is present in the EMBL database under accession number

referred to herein, refers to that in the EMBL database under accession number HSLB2A26, unless stated otherwise or apparent from the context.

Marker 15 is the human PRSM1 protein. The gene for PRSM1 has been cloned and sequenced (Scott et al., Gene 174:135-143 (1996)). These authors identify PRSM1 as a putative metallopeptidase. There is no teaching in this paper of any association with prostate cancer. The sequence of PRSM1 is disclosed in Scott et al. and is present in the EMBL database under accession number HSU58048. For the purpose of this application, the gene and amino acid sequences and sequence positions referred to herein, refers to that in the EMBL database under accession number HSU58048, unless stated otherwise or apparent from the context.

Marker 16 is the human ribosomal large subunit L12 protein. The gene for ribosomal large subunit L12 protein has been cloned and sequenced (Chu et al., Nucleic Acids Res. 21:749-749 (1993)). These authors identify human ribosomal large subunit L12 as a ribosomal protein. There is no teaching in this paper of any association with prostate cancer. The sequence of human ribosomal large subunit L12 is disclosed in Chu et al. and is present in the EMBL database under accession number HSL12A. For the purpose of this application, the gene and amino acid sequences and sequence positions referred to herein, refers to that in the EMBL database under accession number HSL12A, unless stated otherwise or apparent from the context.

Marker 17 is the human 7SK45 RNA. The gene for 7SK45 RNA has been cloned and sequenced (Murphy et al., Nucleic Acids Res. 14:9243-9260 (1986)). These authors identify human 7SK45 as a small cytoplasmic RNA. There is no teaching in this paper of any association with prostate cancer. The sequence of 7SK45 is disclosed in Murphy et al. and is present in the EMBL database under accession number HS7K45. For the purpose of this application, the gene sequences and sequence positions referred to herein, refers to that in the EMBL database under accession numbers: HS7K45, unless stated otherwise or apparent from the context.

Marker 18 is the human KIAA0588 protein (human protocadherin gamma). This protein is abundantly expressed in brain cells. The gene for this protein has been cloned and sequenced (Nagase et al., DNA Research. 5:31-39 (1998)). There is no teaching in this paper of an association with prostate cancer. The sequence of the KIAA0588 protein is present in the EMBL database under accession number AB011160. For the purpose of this application,

application, the gene and amino acid sequences and sequence positions referred to herein, refers to that in the EMBL database under accession number HSSRP14A, unless stated otherwise or apparent from the context.

Marker 23 is bone small proteoglycan I (biglycan) protein. The gene for this protein has been cloned and sequenced (Fisher et al., J. Biol. Chem. 264:4571-4576 (1989)). These authors identify bone small proteoglycan I (biglycan) as a protein derived from human bone-derived cells. There is no teaching in this paper of an association with prostate cancer. The sequence of bone small proteoglycan I (biglycan) protein is disclosed in Fisher et al. and is present in the EMBL database under accession number HSHPGI. For the purpose of this application, the gene and amino acid sequences and sequence positions referred to herein, refers to that in the EMBL database under accession number HSHPGI, unless stated otherwise or apparent from the context.

Marker 24 is the human KIAA0045 gene. The gene for this protein has been cloned and sequenced (Nomura N et al., DNA Res 1:223-229 (1994)). These authors identify KIAA0045 as a protein from the human immature myeloid cell line KG-1. There is no teaching in this paper of an association with prostate cancer. The sequence of KIAA0045 is disclosed in the EMBL database under accession number HSKG1C (accession no. D28476). For the purpose of this application, the gene and amino acid sequences and sequence positions referred to herein, refers to that in the EMBL database under accession number HSKG1C, unless stated otherwise or apparent from the context.

Marker 25 is b-cell receptor associated protein, also known as REA (repressor of estrogen activity). The gene for this protein has been cloned and sequenced (Montano et al., Proc Natl Acad Sci U.S.A. 96:6947-6952 (1999)). These authors identify REA as a protein derived from human breast. There is no teaching in this paper of an association with prostate cancer. The sequence of b-cell receptor associated /REA protein, is disclosed in Montano et al. and is present in the EMBL database under accession number AF150962. For the purpose of this application, the gene and amino acid sequences and sequence positions referred to herein, refers to that in the EMBL database under accession number AF150962, unless stated otherwise or apparent from the context.

Marker 26 is the cystatin B protein. The gene for cystatin B has been cloned and sequenced (Pennacchio et al., Science 271:1731-1734 (1996)). These authors identify cystatin B protein as associated with progressive myoclonus epilepsy disease. There is no teaching in

markers such as PSA, prostatic acid phosphatase, prostate-specific membrane antigen or others (e.g. those reviewed in Gao et al., Prostate 31:264-281 (1977)); markers associated with cell proliferation, invasiveness, tumour angiogenesis, metastasis or any other aspect of carcinogenesis; or markers for other diseases and conditions. Levels of Marker mRNA in the test sample can be detected by any technique known in the art. These include Northern blot analysis, reverse transcriptase-PCR amplification (RT-PCR), microarray analysis and RNAse protection.

In one embodiment, levels of Marker RNA in a sample can be measured in a Northern blot assay. Here, tissue RNA is fractionated by electrophoresis, fixed to a solid membrane support, such as nitrocellulose or nylon, and hybridised to a probe or probes capable of selectively hybridising with the Marker RNA to be detected. The actual levels may be quantitated by reference to one or more control housekeeping genes. Probes may be used singly or in combination. This may also provide information on the size of mRNA detected by the probe. Housekeeping genes are genes which are involved in the general metabolism or maintenance of the cell, and are considered to be expressed at a constant level irrespective of cell type, physiological state or stage in the cell cycle. Examples of suitable housekeeping genes are: beta actin, GAPDH, histone H3.3 or ribosomal protein L13 (Koehler et al., Quantitation of mRNA by Polymerase Chain Reaction. Springer-Verlag, Germany (1995)).

To gauge relative expression levels, a control sample can be run alongside the test sample or, the test result/value can be compared to Marker expression levels expected in a normal or control tissue. These control values can be generated from prior test experiments using normal or control tissues, to generate mean or normal range values for each Marker.

In another embodiment, the Marker nucleic acid in a tissue sample is amplified and quantitatively assayed. The polymerase chain reaction (PCR) procedure can be used to amplify specific nucleic acid sequences through a series of iterative steps including denaturation, annealing of oligonucleotide primers (designed according to the published Marker sequence to be detected), and extension of the primers with DNA polymerase (see, for example, Mullis, et al., U.S. patent No. 4,683,202; Loh et al., Science 243:217 (1988)). In reverse transcriptase-PCR (RT-PCR) this procedure is preceded by a reverse transcription step to allow a large amplification of the number of copies of mRNA (Koehler et al., *supra*). Other known nucleic acid amplification procedures include transcription-based amplification systems (TAS) such as nucleic acid based sequence application (NASBA) and 3SR (Kwoh et

A variety of methods are currently available for making arrays of biological molecules. The 'dot or slot blot' approach, whereby an ordered array of DNA is vacuum blotted using a manifold, or hand blotted by capillary action, onto a porous membrane, such as nylon or nitrocellulose has been around for many years (Maniatis et al., Molecular Cloning-A Laboratory Manual, First Edition, Cold Spring Harbor, (1982)). Methods for preparing a plurality of oligonucleotide sequences and for attaching these to solid supports at high density are also known in the art. For example, US Patent No. 4,562,157 describes a method of using photo-activatable cross-linking groups to immobilise pre-synthesised ligands on surfaces. Fodor et al. (Nature 364:555-556 (1993)) and US Patent No. 5,143,854 describe the 'light-directed chemical synthesis' method for synthesising ligands, including oligonucleotides, directly onto a substrate surface at the desired location. US 5,700,637 also describes methods for *in situ* synthesis of oligonucleotides on solid support surfaces. In addition, such methods for preparing microarrays can easily be automated. International Publication No. WO 95/35505 discloses an automated capillary dispensing device and method for applying biological macromolecules to solid supports. International Publication No. WO 97/44134 also describes devices for delivery of small volumes of liquid (which may contain biological macromolecules) in a precise manner to produce microsized spots on a solid surface to generate a microarray. Similarly, International Publication No. WO 98/10858 also describes an apparatus for the automated synthesis of molecular arrays. Techniques exist for applying the oligonucleotides to the array at high density and for example, techniques exist for applying well in excess of 10^3 distinct polynucleotides per 1 cm^2.

Microarray technology makes it possible to simultaneously study the expression of many thousands of genes in a single experiment. Analysis of gene expression in human tissue (e.g. biopsy tissue) can assist in the diagnosis and prognosis of disease and the evaluation of risk for disease. A comparison of levels of expression of various genes from patients with defined pathological disease conditions with normal patients enables an expression profile, characteristic of disease, to be created. There are currently two main approaches to analyse gene expression using microarrays. In the first approach, cDNA fragments, often generated by PCR, for each of the genes under study are attached to an array. Typically, mRNA isolated from the test samples (i.e. induced or un-induced) is reverse transcribed into cDNA with incorporation of a fluorescent label. The cDNA is sheared and hybridised to the array. If a control test sample is to be run at the same time, mRNA from this sample can be reverse

In transcript profiling, several or many mRNAs are detected in the same procedure. One or more of these mRNAs may be diagnostic of cancer cells (i.e prostate cancer) in a tissue sample. In one embodiment, combinations of probes can be used to classify the cancer cells into clinically relevant types, according to the complex expression pattern of Markers measured on the array. Such classification may help to define which tumours are growing aggressively, or harbour latent signs of aggression, or are less aggressive or benign. The array provides a quantitative measure of Marker RNAs. This is done by comparison of Marker RNA signal with control signal. In a preferred embodiment hybridisation signals generated are measured by computer software analysis of images on phosphorimage screens exposed to radioactively labelled tissue RNA hybridised to a microarray of probes on a solid support such as a nylon membrane. In another, quantities are measured by densitometry measurements of radiation-sensitive film (e.g. X-ray film), or estimated by visual means. In another embodiment quantities are measured by use of fluorescently labelled probe, which may be a mixture of tumour and normal RNA differentially labelled with different fluorophores, allowing quantities of Marker mRNAs to be expressed as a ratio versus the normal level. The solid support in this type of experiment is generally a glass microscope slide, and detection is by fluorescence microscopy and computer imaging.

The detection of specific interactions may be performed by detecting the positions where the labelled target sequences are attached to the array. Radiolabelled probes can be detected using conventional autoradiography techniques. Use of scanning autoradiography with a digitised scanner and suitable software for analysing the results is preferred. Where the label is a fluorescent label, the apparatus described, e.g. in International Publication No. WO 90/15070, US Patent No. 5, 143,854 or US Patent No. 5,744,305 may be advantageously applied. Indeed, most array formats use fluorescent readouts to detect labelled capture:target duplex formation. Laser confocal fluorescence microscopy is another technique routinely in use (M.J.Kozal et al., Nature Medicine 2:753-759 (1996)). Mass spectrometry may also be used to detect oligonucleotides bound to a DNA array (Little et al, Analytical Chemistry 69: 4540-4546, (1997)). Whatever the reporter system used, sophisticated gadgetry and software may be required in order to interpret large numbers of readouts into meaningful data (such as described, for example, in US Patent No. 5,800,992 or International Publication No. WO 90/04652).

RNA can be extracted at a later date. Proprietary reagents are available which allow tissue or cells to be conveniently stored for several days at room temperature and up to several months at 4°C (e.g. RNAlater, Ambion Inc., TX). Prior to extraction, methods such as grinding, blending or homogenisation are used to dissipate the tissue in a suitable extraction buffer. Typical protocols then use solvent extraction and selective precipitation techniques. Example 1 describes such a method.

In another embodiment, tissue is directly analysed for the presence of Marker nucleic acid. This can be by *in situ* hybridisation, where sections of tissue may be interrogated with specific probes to determine which morphological cell type in the sample displays a marker nucleic acid, such as sequences corresponding to Markers 1-26. *In situ* hybridisation typically comprises 3 steps. Firstly tissue is fixed, and sections are prepared by standard treatments known to those in the art (Polack and McGee, *In situ* hybridisation: principles and practice. Oxford University Press, 1998). Secondly, Marker mRNA and amplified Marker DNA can be detected by hybridisation with e.g. a biotin-, digoxygenin- or radio-labelled Marker probe, typically for 2-16 hours at 42°C in a suitable hybridisation buffer. A typical buffer might contain 50% formamide, 5% dextran sulphate, 2xSSC and 10-20ng of probe per 7µl (Herrington and McGee, Diagnostic Molecular Pathology. IRL Press, Oxford, 1992). The probe can be made of DNA or RNA. Lastly, following stringency washes, the probes in hybridisation complexes are detected with chromogenic or fluorescent reagents, which can be visualised by microscopy, or by autoradiography in the case of radiolabelled probes. Signal amplification systems using e.g. tyramide can be used to increase sensitivity (Polack and McGee, *supra)*. *In situ* PCR using oligonucleotide probes complementary to the nucleic acid of any of Markers 1 - 26 is therefore envisaged. *In situ* hybridisation can follow *in situ* PCR, giving greater specificity (Polack and McGee, *supra*). Techniques for quantitation of signal, and quantification of positive cells in a section are available to the pathologist using image analysis. *In situ* Marker visualisation permits localisation of signal in mixed-tissue specimens commonly found in tumours, and is compatible with many histological staining procedures. In one embodiment, several probes can be differentially labelled and hybridised simultaneously to the same section, and detected using appropriate reagents. In another, serial sections from the same sample can be analysed with a panel of probes. Quantitation may involve comparison with one or more control housekeeping genes as discussed above.

cDNA) then the probe or primer sequence can hybridise to the sense or antisense strand. If however the target is mRNA (single stranded sense strand) the primer/probe sequence will have to be the antisense complement.

An example of a suitable hybridisation solution when a nucleic acid is immobilised on a nylon membrane and the probe nucleic acid is greater than 500 bases or base pairs is: 6 x SSC (saline sodium citrate), 0.5% SDS (sodium dodecyl sulphate), 100µg/ml denatured, sonicated salmon sperm DNA. The hybridisation being performed at 68°C for at least 1 hour and the filters then washed at 68°C in 1 x SSC, or for higher stringency, 0.1 x SSC/0.1% SDS.

An example of a suitable hybridisation solution when a nucleic acid is immobilised on a nylon membrane and the probe is an oligonucleotide of between 12 and 50 bases is: 3M trimethylammonium chloride (TMACl), 0.01M sodium phosphate (pH 6.8), 1mM EDTA (pH 7.6) , 0.5% SDS,100µg/ml denatured, sonicated salmon sperm DNA and 0.1 dried skimmed milk. The optimal hybridisation temperature (Tm) is usually chosen to be 5°C below the Ti of the hybrid chain. Ti is the irreversible melting temperature of the hybrid formed between the probe and its target. If there are any mismatches between the probe and the target, the Tm will be lower. As a general guide, the recommended hybridisation temperature for 17-mers in 3M TMACl is 48-50°C; for 19-mers,it is 55-57°C; and for 20-mers, it is 58-66°C.

A suitable hybridisation protocol is described in Example 5, however, operable variations to this method will be apparent to the person skilled in the art.

Where the cDNA molecules of Markers 1 - 26 encode proteins or parts of cellular proteins, these may themselves act as prostate disease Markers. To detect proteins in tissue, cells, body fluids or extracts of these sample types, specific antibody can be used. These antibodies can be prepared using the Marker protein/polypeptides.

Methods of making and detecting labelled antibodies are well known (Campbell; Monoclonal Antibody Technology, in: Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13. Eds: Burdon R et al. Elsevier, Amsterdam (1984)). The term antibody includes both monoclonal antibodies, which are a substantially homogeneous population, and polyclonal antibodies which are heterogeneous populations. The term also includes inter alia, humanised and chimeric antibodies. Monoclonal antibodies to specific antigens may be obtained by methods known to those skilled in the art, such as from hybridoma cells, phage display libraries or other methods. Monoclonal antibodies may be inter alia, human, rat or

that the nucleic acid sequence encoding the polypeptide is transcribed into RNA in the host cell transformed or transfected by the expression vector construct. The coding sequence may or may not contain a signal peptide or leader sequence for secretion of the polypeptide out of the host cell. Expression and purification of the Marker polypeptides can be easily performed using methods well known in the art (for example as described in Sambrook et al. *supra*).

The Marker polypeptides so produced can then be used to inoculate animals, from which serum samples, containing the specific antibody against the introduced Marker protein/polypeptide, can later be obtained.

Rodent antibodies may be humanised using recombinant DNA technology according to techniques known in the art. Alternatively, chimeric antibodies, single chain antibodies, Fab fragments may also be developed against the polypeptides of the invention (Huse et al., Science 256:1275-1281 (1989)), using skills known in the art. Antibodies so produced have a number of uses which will be evident to the molecular biologist or immunologist skilled in the art. Such uses include, but are not limited to, monitoring enzyme expression, development of assays to measure enzyme activity and use as a therapeutic agent. Enzyme linked immunosorbant assays (ELISAs) are well known in the art and would be particularly suitable for detecting the Marker proteins or polypeptide fragments thereof in a test sample.

The expression system described in Example 3 can produce protein for use as an antigen for the generation of antibodies for use in an ELISA assay to detect Marker protein in body fluids or by immunohistochemistry (as described in Example 4) or other means. In addition, an antibody could be used individually or as part of a panel of antibodies, together with a control antibody which reacts to a common protein, on a dipstick or similar diagnostic device.

Levels of Marker gene expression can also be detected by screening for levels of polypeptide (Marker protein). For example, monoclonal antibodies immunoreactive with a Marker protein can be used to screen a test sample. Such immunological assays can be done in any convenient format known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Functional assays can also be used, such as protein binding determinations.

In another preferred embodiment antibodies directed against a Marker protein or proteins can be used, to detect, prognose, diagnose and stage prostate cancer or its precursor lesions, or related prostate disorders. Various histological staining methods known in the art,

Thus, according to another aspect of the invention there is provided a method for treating a patient suffering from a prostate disorder comprising administering to said patient an effective amount of an anti-sense molecule capable of binding to the mRNA of a Marker gene selected from the group consisting of: Marker 1 - Marker 26, and inhibiting expression of the protein product of the Marker gene.

Complete inhibition of protein production is not essential, indeed may be detrimental. It is likely that inhibition to a state similar to that in normal tissues would be desired.

This aspect of antisense therapy is particularly applicable if the prostate disorder is a direct cause of over-expression of the Marker gene(s) in question, although it is equally applicable if said Marker gene(s) indirectly cause the prostate disorder. Having identified the particular Marker genes (1 - 26) over-expressed in prostate disorders, and with knowledge of the gene and mRNA sequence the person skilled in the art is able to design suitable antisense nucleic acid therapeutic molecules and administer them as required.

Antisense oligonucleotide molecules with therapeutic potential can be determined experimentally using well established techniques. To enable methods of down-regulating expression of a Marker gene of the present invention in mammalian cells, an example antisense expression construct can be readily constructed for instance using the pREP10 vector (Invitrogen Corporation). Transcripts are expected to inhibit translation of the gene in cells transfected with this type of construct. Antisense transcripts are effective for inhibiting translation of the native gene transcript, and capable of inducing the effects (e.g., regulation of tissue physiology) herein described. Oligonucleotides which are complementary to and hybridisable with any portion of Marker gene mRNA are contemplated for therapeutic use. U.S. Patent No. 5,639,595, "Identification of Novel Drugs and Reagents", issued Jun. 17, 1997, wherein methods of identifying oligonucleotide sequences that display *in vivo* activity are thoroughly described, is herein incorporated by reference. Expression vectors containing random oligonucleotide sequences derived from Marker polynucleotides are transformed into cells. The cells are then assayed for a phenotype resulting from the desired activity of the oligonucleotide. Once cells with the desired phenotype have been identified, the sequence of the oligonucleotide having the desired activity can be identified. Identification may be accomplished by recovering the vector or by polymerase chain reaction (PCR) amplification and sequencing the region containing the inserted nucleic acid material. Antisense molecules can be synthesised for antisense therapy. These antisense molecules may be DNA, stable

analogous to quantitative RT-PCR already described. Marker gene amplification could also be detected *in situ* in cells by probe hybridisation. Fluorescently-labelled probes (used in Fluorescent in situ Hybridisation or FISH) are ideal for this purpose as they allow subnuclear localisation via microscopy, and permit estimates of gene copy number by subnuclear spot intensity or number (Mark et al. Exp Mol Pathol 66:170-8 (1999)). Quantitative analysis of DNA for amplifications of Marker genes can also be carried out by Southern analysis, a method which is widely known to those skilled in the art (Sambrook et al., *supra*). DNA can be extracted from clinical material using established methods (Sambrook et al., *supra*). The methods of the invention can therefore also be directed to measuring genomic DNA levels of one or more of the identified Markers.

If a Marker mRNA encodes a secreted protein, that protein is likely to be present in body fluids. Proteins, especially secreted proteins, can be quantified in blood, serum, urine, semen and other fluids. Specific antibodies can often detect abundant proteins in ELISA tests on body fluid samples without enrichment. Prostate-specific antigen (PSA) falls into this category. PSA is an important Marker produced by prostatic epithelial cells and almost always expressed in prostate cancer, though not exclusively so. Clinically significant levels of proteins such as PSA are defined by appropriate studies, and protein levels are typically given as ng per mL of sample. Serum protein tests with greater specificity for cancer are needed. Detection of rare proteins may require that the protein is concentrated by e.g. precipitation. Thus, in a further embodiment of the invention diagnosis or prognosis or prostate disorder, or stage monitoring of the prostate disorder or therapeutic efficacy assessment is performed by testing for aberrant levels of one or more Marker proteins of the invention, which Marker protein is a secreted protein, in a bodily fluid.

The inventors predict that Markers 9, 19 and 24 (at least) are secreted proteins.

All the essential materials and reagents required for detecting or monitoring prostate disorder Markers in a test sample may be assembled together in a kit. Such a kit may comprise one or more diagnostic cDNA probes or oligonucleotide primers together with control probes/primers. The kit may contain probes immobilised on a microarray substrate such as a filter membrane or silicon-based substrate. The kit may also comprise samples of total RNA derived from tissues of various physiological states, such as normal, BPH, confined tumour and metastatic tumour, for example, to be used as controls. The kit may also comprise appropriate packaging and instructions for use in the methods of the invention.

Elmer 7700). Preferred oligonucleotide primers for detection of Marker mRNAs are selected from Markers 1-26.

In another embodiment the kit comprises one or more antibodies specific for one or other of the Markers identified herein for use in immunohistochemical analysis.

In another embodiment the kit is an ELISA kit comprising one or more antibodies specific for one or other of the Markers identified herein.

In another aspect of the invention, one or more of the 26 Markers can also be used in biochemical assays to identify agents which modulate the activity of the Marker proteins. The design and implementation of such assays will be evident to the biochemist of ordinary skill. The protein, particularly if it is a biochemical enzyme, may be used to turn over a convenient substrate whilst incorporating or losing a labelled component to define a test system. Test compounds are introduced into the test system and measurements made to determine their effect on enzyme activity. Such assays are useful to identify inhibitors of the enzyme which may prove valuable as therapeutic agents.

The inventors believe that Marker 19 (Factor V) is an example of a suitable biochemical enzyme that can be used in a suitable biochemical assay to identify modulators.

In a further aspect of the invention, each of the Markers can be used to characterise cell cultures in a screen for therapeutic agents, such as a high throughput screen. Effects of test compounds may be assayed by changes in mRNA or protein of any of Markers 1-26. As described above, cells (i.e. mammalian, bacterial etc) can be engineered to express one of the Markers identified herein.

Thus, according to a further aspect of the invention there is provided a method of testing potential therapeutic agents for the ability to suppress a prostate disorder phenotype comprising contacting a test compound with a cell engineered to express one of the Markers identified herein; and determining whether said test compound suppressed expression of the Marker.

Thus, according to a further aspect of the invention there is provided a screening assay or method for identifying potential anti-prostate disorder therapeutic compounds comprising contacting an assay system capable of detecting the effect of a test compound against expression levels of one or more of the Markers selected from the group consisting of: Marker 1 - Marker 26, with a test compound and assessing the change in expression level of the particular Marker under study.

Further features of the invention include:

A method of treatment of a patient suffering from a prostate disorder, comprising administration to the patient of a compound capable of reducing the transcription or expression of any one of Markers 1 - 26.

A method of treatment of a patient suffering from a prostate disorder, comprising administration to the patient an antisense nucleic acid molecule targeted against the mRNA of any one of Markers 1 - 26.

Use of an antisense nucleic acid molecule or an antibody directed against any one of Markers 1 - 26, in the manufacture of a medicament for treating a prostate disorder.

Each aspect of the invention involves detection or use of one or more of Markers 1 - 26. A preferred sub-group of Markers are those selected from the group consisting of Markers: 8, 13, 14, 15, 17, 18, 19, 20, 22, 24, 25 and 26.

The invention will be further described by way of the following non-limiting examples and figures in which data illustrating over-expression of markers is included:

Figure 1. Filter microarray data showing over-expression of Markers 1- 18 and 20-25 in prostate cancer relative to normal prostate. Overexpression value for Marker 26 (not shown) was found to be at the same level as Marker 13. Values given are mean expression level for 9 prostate cancer samples in those samples where over-expression of 1.5-fold or more was detected. Expression is given relative to normal prostate level (i.e. compared to the mean of 3 normal prostate datasets. These 3 datasets comprised 2 different RNA mixtures, each with at least 10 normal sample components).

Figure 2. Filter microarray data showing over-expression of Markers 1-5, 7-12, 17, 18, 20 and 23 in prostate cancer relative to BPH. Values given are mean expression level for 9 prostate cancer samples in those samples where over-expression of 1.5-fold or more was detected. Expression is given relative to BPH level (i.e. compared to the mean of 13 BPH datasets).

Example 1: Identification and Evaluation of Markers of Prostate Disease by cDNA Microarray Analysis

The microarray analysis protocol described in the following example was developed as a means to determine the relative abundances of mRNA species that are expressed in various tissues. Microarray analysis was used to identify differentially expressed RNA species

treated with DNAse I (also Gibco BRL), again as recommended by the manufacturer. For 1st strand label synthesis, 20µg total RNA was reverse transcribed into cDNA in the presence of radiolabelled nucleotide (^{33}P), using Superscript II enzyme (Gibco BRL) according to manufacturer's instructions. Labelled RNA was purified using GFX columns (Amersham Pharmacia Biotech, St. Albans, UK) according to manufacturers instructions, and added to 10 ml/filter of Church hybridisation buffer described below.

Prior to hybridisation, filters were wetted briefly in 2xSSC, then incubated in 5ml prewarmed Church hybridisation solution (0.5M sodium phosphate, 7% SDS, 1mM EDTA, pH 7.2) at 65°C for 2-6 hours. Probe was denatured at 100°C for 5 min, placed on ice for at least 5 min, then added to 5ml fresh Church hybridisation buffer in the presence of the filter, and mixed by swirling the bottle with the lid on for even probe distribution. Hybridisation was carried out at 65°C for 12-16 hours. Filters were rinsed briefly in pre-warmed Church wash solution (40mM sodium phosphate, 1% SDS, pH 7.2), then incubated twice in the same solution at 65°C for 20 min. Filters were drained briefly, wrapped in Saran wrap (Dow Chemical Company, USA) and exposed to phosphor screens for 3-6 days (screens and cassettes supplied by Molecular Dynamics, CA). The screens were then scanned using a Storm 830 phosphorimager (Molecular Dynamics). Array Vision software (Incyte Pharmaceuticals, CA, USA) was used to visualise the hybridisation images and generate quantitative numbers for each spot.

Typically, for comparison of data from different arrays, data is generated as a value relative to an internal standard for each array. Following export of ArrayVision data into Microsoft Excel format, spot measurements for data were normalised to housekeeping gene hybridisation signals known to be constant or relatively constant, using a simple Excel macro. A total of 38 spot values (from 16 different clones) representing 12 different housekeeping genes were used to generate an average housekeeping measurement. The gene and EMBL accession number of the housekeeping genes used are listed in Table 1. Background values were not subtracted, but to eliminate spurious low-level signals indistinguishable from noise, a local background value from 3 blank spots out of every 7x7 array was taken, and every value <2-fold this value was ignored.

To generate representative values for normal prostate, data from 3 array hybridisation experiments to normal prostate RNA was normalised to the mean housekeeping gene value, then the mean of these 3 values was taken as the control dataset.

histopathological analysis. **Table 2** lists the 26 genes we have identified as being Markers for prostatic disease.

Control number	Gene	NCBI genInfo identifier	Spots on Array
1	Human ribosomal protein S14 gene	g337498	2
2	Human ribosomal protein S14 gene	g337498	2
3	Human acidic ribosomal phosphoprotein P0	g190231	2
4	Human alpha-tubulin mRNA	g340020	2
5	Human mRNA for TEF-5 protein	g1848081	2
6	Human transcriptional enhancer factor	g339440	2
7	Human transcription factor RTEF-1	g1561727	2
8	Human hypoxanthine phosphoribosyltransferase	g184349	2
9	Human hypoxanthine phosphoribosyltransferase	g184349	2
10	Human mRNA for transcription factor, TEF3	g1403337	2
11	Human acidic ribosomal phosphoprotein P1	g190233	2
12	Human mRNA for ribosomal protein L19	g36127	4
13	Human hH3.3B gene for histone H3.3	g761715	4
14	Human pancreatic phospholipase A-2 (PLA-2)	g190008	2
15	Human mRNA for ribosomal protein L19	g36127	4
16	Human acidic ribosomal phosphoprotein P1	g190233	2

Table 1. Names and identities of control housekeeping genes used to normalise microarray data. This set was identified from a wider set, by comparison of variability of candidate housekeeping gene signals over 13 filter hybridisation experiments as a group showing minimal variability.

prostate cancer compared to BPH, or in the case of Marker 19, not detected at all in normal prostate. In addition, Markers 6, 13, 14 and 25 were not detected in BPH.

Example 2: Quantitative RT-PCR test

RT-PCR is a convenient method for assaying the relative abundance of mRNA species expressed in various cells, tissues and organs.

Reproducibly accurate amplification of an mRNA can be achieved by firstly synthesising a DNA template from the mRNA using a reverse transcriptase, then by PCR using a heat-stable DNA polymerase (e.g. Taq polymerase). An optimised amplification protocol is essential for quantitation of nucleic acids by PCR because small differences in efficiency of the reaction can greatly influence the reaction rates, with a subsequent effect on PCR yield. Optimisation of PCR is not usually problematic (refer to: Koehler *supra,* and references therein). Quantitation of RT-PCR products can be done while the reaction products are building up exponentially, preferably following each round of amplification (known as real-time PCR). For quantitation, analysis is carried out by reference to one or more housekeeping genes which are also amplified by RT-PCR, often in a different reaction tube. Quantitation of RT-PCR product may be undertaken, for example, by gel electrophoresis visual inspection or image analysis, HPLC (Koehler et al., *supra*) or by use of suitable detection methods such as described above.

In practise, specific pairs of oligonucleotide primers corresponding to Markers 1-26 can be used to trigger amplification of specific marker mRNAs. The Marker mRNA is quantitated relative to a standard housekeeping mRNA using a real-time RT-PCR assay. For the purpose of this example, the housekeeping gene is ribosomal protein L19, but any such gene could be used.

Firstly, RNA is isolated from a clinical or tissue sample as in example 1. Aliquots of RNA are used in a reverse transcription reaction using random hexamers or oligo-dT as primers, and for example Moloney Murine Leukaemia Virus (MuLV) or Avian Myeloblastosis Virus (AMV) reverse transcriptase. A typical 20ml reverse transcription reaction comprises the following: 10 mM each dNTP, 50 mM Tris-HCl, pH8.3, 50 mM KCl, 50 mM dithiothrietol, 0.5 mM spermidine, 10ng oligo-dT primer, up to 1µg RNA, dissolved in DEPC-treated water. This mixture is incubated at 65°C for 15 min, then chilled on ice for 5 min. 5 units of AMV reverse transcriptase and 10 units of RNAse inhibitor are added. The

Example 3: Generation of antibody specific to Protein encoded by Markers 1-26

Antibody specific to Markers 1-26 could be used to detect protein products of these Markers in tissue samples and body fluids. Ideally for diagnosis, easily accessible samples are required, involving minimally invasive techniques. Typically, these samples include blood, plasma, serum, saliva, urine and semen. These fluids are far removed from prostate cells and, in a normal individual, would not be expected to contain mRNA of prostatic origin. However, protein may be transported across cell membrane barriers into body fluids. Prostate-specific antigen is one such protein, and is easily assayed by a simple blood test using specific antibody against the protein. For new markers, e.g. Markers 1-26, it may be necessary to generate novel antibody. This example describes a method of generating protein in vitro, which can then be used to raise antibody.

One way to generate protein is to express the cloned gene in *E. coli*. This requires that the cDNA, e.g. Markers 1-26 is cloned into a vector capable of expressing the encoded protein in the bacterial host. Optionally, it may be desirable to incorporate a molecular tag into the protein so that it can be easily purified. One such tag in wide use is the 6xHis tag. A protein with this tag is easily isolated from a cell culture extract by affinity chromatography, and can be used in relatively pure form to inoculate an animal for antibody generation.
Having subcloned the cDNA or cDNA fragment into an appropriate vector, e.g. pHAT10, 11, 12 or 20 (Clontech, Palo Alto, CA) and confirmed the integrity of the insert such as sequence and orientation. The subclone is cultured on a suitable scale, cells are harvested by centrifugation and then lysed, and the protein extract incubated with TALON resin according to manufacturer's instructions (Clontech, Palo Alto, CA). The resin is washed and recombinant protein is eluted by adjusting the pH or imidazole concentration. If desired, the 6xHIS tag can be enzymatically removed using a specific protease (Clontech). To generate a specific antibody, the purified protein is injected into a host animal, usually rabbit, sheep or goat. After boost injections, the serum is periodically collected and tested for antibody. Polyclonal antibody can be purified form the serum using standard techniques, or the animal's spleen can be harvested for the production of hybridomas. Techniques for the production of antibodies, both polyclonal and monoclonal, are well known to those skilled in the art (Catty D (ed.) Antibodies: A Practical Approach. Vol 1 (1988), Vol2 (1989)).

A hybridisation probe can be generated from a synthetic oligonucleotide or a dephosphorylated restriction fragment sequence by addition of a radioactive 5' phosphate group from [γ-^{32}P]ATP by the action of T4 polynucleotide kinase. 20 pmoles of the oligonucleotide are added to a 20µl reaction containing 100mM Tris, pH7.5 , 10mM $MgCl_2$, 0.1mM spermidine, 20mM dithiothreitol (DTT), 7.55µM ATP, 55µCi [γ-^{32}P]ATP and 2.5u T4 polynucleotide kinase (Pharmacia Biotechnology Ltd, Uppsala, Sweden). The reaction is incubated for 30 minutes at 37°C and then for 10 minutes at 70°C prior to use in hybridisation. Methods for the generation of hybridisation probes from oligonucleotides or from DNA and RNA fragments are described in Chapters 11 and 10 respectively in Sambrook et al. (*ibid*). A number of proprietary kits are also available for these procedures.

Filter preparation

The sample DNA could be isolated and run on an agarose gel and Southern blotted onto a nitrocellulose or nylon filter using standard techniques.

Hybridisation conditions

Filters containing the nucleic acid are pre-hybridised in 100ml of a solution containing 6x SSC, 0.1%SDS and 0.25% dried skimmed milk (Marvel™) at 65°C for a minimum of 1 hour in a suitable enclosed vessel. A proprietary hybridisation apparatus such as model HB-1 (Techne Ltd) provides reproducible conditions for the experiment.

The pre-hybridisation solution is then replaced by 10ml of a probe solution containing 6xSSC, 0.1% SDS, 0.25% dried skimmed milk (e.g. Marvel™) and the oligonucleotide probe generated above. The filters are incubated in this solution for 5 minutes at 65°C before allowing the temperature to fall gradually to below 30°C. The probe solution is then discarded and the filters washed in 100ml 6xSSC, 0.1% SDS at room temperature for 5 minutes. Further washes (1-3) are then made in fresh batches of the same solution at 30°C and then, optionally, in 10°C increments up to 60°C for 5 minutes per wash.

After washing, the filters are dried and used to expose an X-ray film such as Hyperfilm™ MP (Amersham International) at -70°C in a light-tight film cassette using a fast tungstate intensifying screen to enhance the photographic image. The film is exposed for a suitable period (normally overnight) before developing to reveal the photographic image of the radio-active areas on the filters. Related nucleic acid sequences are identified by the presence of a photographic image compared to totally unrelated sequences which should not

then chilled quickly on ice-water, is placed on the tissue section and a coverslip is sealed on to the slide using rubber solution. A suitable probe is a digoxygenin-labelled 300-600 base pair cDNA clone or fragment thereof, corresponding to one of Markers 1-26, prepared by one of several methods known to the art. The slides are then incubated in a sealed chamber for 16h at 37°C. The rubber solution is peeled away and coverslips are removed by immersion of the slides in 2x SSC, 0.1% SDS. Slides are washed 4 times for 5 min each at room temperature, and twice for 10 min at 65°C, in 2x SSC, 0.1% SDS, and rinsed briefly in 2x SSC. Hybridisation is detected as follows: Nonspecific binding is blocked by incubating the slides in blocking buffer (0.1M Tris-HCL pH 7.5, 0.1M NaCl, 2mM $MgCl_2$, 3% bovine serum albumin) for 10 min. Slides are then flooded with anti-digoxygenin-conjugated alkaline phosphatase (1/500 dilution of stock; Boehringer, Mannheim, Germany) in blocking buffer, and incubated 2 h at room temperature, then washed in blocking buffer 3 times for 3 min each. Slides are placed in buffer 2 (0.1 M Tris-HCl pH 9.5, 0.1 M NaCl, 50 mM $MgCl_2$) for 10 min, then immersed in substrate buffer (100mL buffer 2 containing 25 mg levamisole, plus 35 mg nitroblue tetrazolium chloride dissolved in 277 mL 70% dimethylformamide, plus 17 mg 5-bromo-4-indoyl-phosphate dissolved in 222 mL 100% dimethylformamide) for 10-30 min in the dark. The slides are then immersed in 20 mM Tris-HCl pH7.5, 5 mM EDTA for 5 min, rinsed with tap water for 5-10 min, mounted in aqueous mountant and examined under a microscope.

Example 7. Screening for therapeutic agents capable of inhibiting expression of any of Markers 1-26.

Markers 1-26 may be employed in a process for screening compounds which either inhibit, promote or modulate the expression of Markers 1-26. Examples of potential Marker 1-26 agonists are small molecules such as organic molecules or peptides, antibodies or oligonucleotides which bind to Markers 1-26 and inhibit expression or activity. One assay for therapeutic agents uses cultured cells, and measures transcript abundance using a microarray as described in Example 1. Typical prostate cell lines such as LNCaP, PC-3 and DU145 are available from the European Collection of Cell Cultures, Salisbury UK. Cell culture is a standard technique well known to those practised in the art. Briefly, in one suitable example, a seed vial of LNCaP cells is inoculated into 500mL culture flasks containing 20-50ml RPMI medium containing 10% fetal calf serum and 1% glutamate (all components Gibco, UK).

Claims:

1. A method for diagnosing or prognosing or monitoring a prostate disorder comprising testing a biological sample from an individual for aberrant levels of one or more of the Markers selected from the group consisting of: Marker 1 to Marker 26.

2. A method as claimed in claim 1, wherein the Marker(s) are selected form the group consisting of Markers: 8, 13, 14, 15, 17, 18, 19, 20, 22, 24, 25 and 26.

3. A method for distinguishing prostate cancer from BPH comprising testing a biological sample for aberrant levels of one or more of the Markers selected from the group consisting of Marker 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 17, 18, 20, 23, 25 and 26.

4. A method as claimed in any of claims 1-3 wherein mRNA transcript levels and/or protein levels of the Marker(s) is/are measured.

5. A method as claimed in claim 4, wherein mRNA transcript levels are measured using reverse-transcriptase polymerase chain reaction (RT-PCR).

6. A method for measuring Marker mRNA transcript levels as claimed in claim 4, which method involves the use of one or more oligonucleotide probes each capable of selectively hybridising to nucleic acid of a Marker of interest to determine the expression level of said Marker of interest.

7. A method for measuring Marker protein levels as claimed in claim 4, which method involves the use of one or more antibodies each capable of selectively binding to a Marker protein or protein fragment of interest to determine the expression level of said Marker of interest.

8. Use of an antibody selective for a Marker protein selected from the group consisting of: Marker 1 - Marker 26, in an assay to monitor therapeutic efficacy.

9. A diagnostic kit for diagnosing or prognosing or monitoring a prostate disorder comprising, one or more diagnostic probe(s) and/or one or more diagnostic primer(s) and/or one or more antibodies capable of selectively hybridising or binding to one or more of the Markers 1 -26.

10. A screening assay for identifying potential anti-prostate disorder therapeutic compounds comprising contacting an assay system capable of detecting the effect of a test compound on expression levels of one or more of the Markers selected from the group consisting of: Marker 1 - Marker 26, with a test compound and assessing the change in expression level of the particular Marker under study.

11. A method of testing potential therapeutic agents for the ability to suppress a prostate disorder phenotype comprising contacting a test compound with a cell engineered to express one of the Markers identified herein; and determining whether said test compound suppressed expression of the Marker.

12. A compound or agent identified by the screening assay according to claim 10 or the method according to claim 11.

13. A method of treatment of a patient suffering from a prostate disorder, comprising administration to the patient of a compound capable of reducing the transcription or expression of any one of Markers 1 - 26.

Figure 1

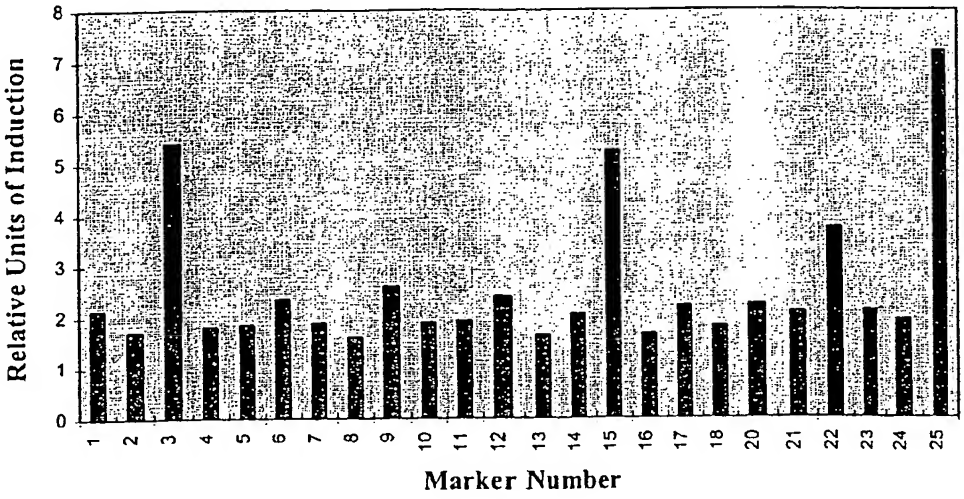
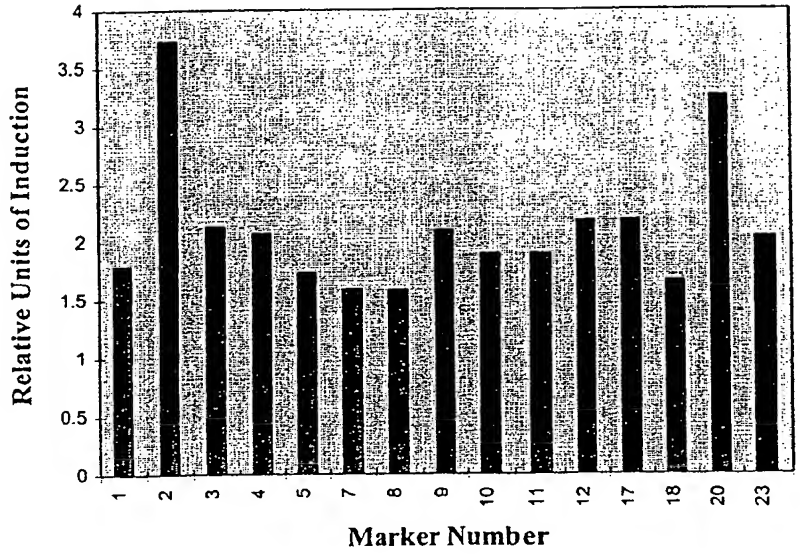


Figure 2



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